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Intraoperative fluorescence imaging in cancer

Crane, Lucia Marie Albertine

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SELECTING POTENTIAL
TARGETABLE BIOMARKERS
FOR IMAGING PURPOSES
IN COLORECTAL CANCER
USING TARGET SELECTION
CRITERIA (TASC):
A NOVEL TARGET
IDENTIFICATION TOOL

MARLEEN VAN OOSTEN
LUCIA M.A. CRANE
JOOST BART
FIJS W. VAN LEEUWEN
GOOITZEN M. VAN DAM

ABSTRACT

Peritoneal carcinomatosis (PC) of colorectal origin is associated with a poor prognosis. However, cytoreductive surgery combined with hyperthermic intraperitoneal chemotherapy (HIPEC) is available for a selected group of PC-patients, which significantly increases overall survival rates up to 30%. As a consequence, there is substantial room for improvement.

Tumor-targeting is expected to improve the treatment efficacy of colorectal cancer (CRC) further through; I) more sensitive pre-operative tumor detection, thus reducing overtreatment; II) better intra-operative detection and surgical elimination of residual disease using tumor-specific intra-operative imaging; and III) tumor-specific targeted therapeutics. This review focuses in particular on the development of tumor-targeted imaging agents. A large number of biomarkers are known to be upregulated in CRC. However, to date no validated criteria have been described for selection of the most promising biomarkers for tumor-targeting. Such a scoring system might improve the selection of the correct biomarker for imaging purposes.

In this review, we present the TArget Selection Criteria (TASC) scoring system for selection of potential biomarkers for tumor-targeted imaging. By applying TASC to biomarkers for CRC, we identified seven biomarkers (CEA, CXCR4, EGFR, EpCAM, MMPs, MUC1 and VEGF-A) that seem most suitable for tumor-targeted imaging applications in colorectal cancer. Further cross-validation studies in CRC and other tumor types are necessary to establish its definitive value.

INTRODUCTION

Patients with colorectal cancer (CRC) have an estimated 5-year survival varying from approximately 90% in patients with stage I disease (Dukes A) to approximately 10% in patients with metastatic disease (Dukes D).¹ Peritoneal carcinomatosis (PC) is a common form of end-stage colorectal cancer (CRC), affecting 10-15% of patients at the time of primary surgery and accounting for 25-35% of the recurrences of CRC.² PC has a median survival of 5-7 months without treatment.³⁻⁵

Since the last decade, selected stage IV CRC patients with PC are treated with hyperthermic intraperitoneal chemotherapy (HIPEC). This procedure consists of flushing the intra-abdominal cavity with heated chemotherapy peri-operatively after primary cytoreduction. HIPEC improves the median survival to 13-63 months, with a 5-year survival varying from 19-51%.⁶⁻¹⁰ However, further improvement is still desirable.

A more extensive surgical cytoreduction is associated with an increase in survival.^{11, 12} Furthermore, because penetration of chemotherapeutic drugs into peritoneally located tumor tissue is only superficial (limited to 1-2 mm), optimal cytoreduction by removing all visible tumor noduli is an essential prerequisite for the HIPEC procedure.¹³⁻¹⁵

The limited survival in stage IV CRC asks for a more vigorous approach in order to improve prognosis. Current research is mainly focused on tumor-targeted imaging and therapy for diagnosis, treatment and follow-up, as these are expected to yield tumor-specific and thus stronger diagnostic and therapeutic effects. Therefore, objective identification of suitable tumor-biomarkers for diagnostic and therapeutic purposes seems appropriate. Furthermore, tumor-targeted imaging can aid in identification of metastatic disease and in detection of recurrent disease. In this review, we emphasize tumor-targeted imaging, as targeted therapeutics demand an entirely different approach for a meta-analysis.

A large number of biomarkers have been reported to play an important role in CRC. However, a limited number of these markers are suitable for tumor-targeting based on characteristics such as for example expression rates. In literature, little objective data is available on how to determine the suitability of a potential target. Therefore, we set out to design a novel scoring system for classification and selection of biomarkers for tumor-targeting applications. CRC is used as a clinical example for development and initial testing of this novel scoring system. With the emphasis on diagnostic and intra-operative imaging, we identified the most promising markers for tumor-targeting in CRC using the scoring system.

In conclusion, in this review, we provide an overview of potential biomarkers for tumor-targeting in CRC, supported by a newly designed Target Selection Criteria (TASC) scoring system.

METHODS

DESIGN OF THE TARGET SELECTION CRITERIA (TASC) SCORING SYSTEM

Seven most important target characteristics selected based on literature were summarized and granted 0-6 points, in order of importance. Subsequently, the selection system was tested by scoring a number of random biomarkers. Cut-off values were determined and the scores were slightly adjusted where necessary to assure realistic outcomes. Finally, the selection system was further validated by testing a wide spectrum of biomarkers on the basis of a publication of Cardoso et al.¹⁶

LITERATURE SEARCH METHODS

Cardoso et al.¹⁶ presented a table of genes found to be upregulated in CRC compared to normal colon tissue, as confirmed in 3 or more articles. The initial literature search query was based on this extensive table of genes. Additionally, based on this table, we analysed all genes mentioned for overexpression of the related protein, as protein expression is not always synchronously upregulated, using Swissprot and Pubmed from 1985-May 2010 (Figure 1). Furthermore, we included a number of proteins that were not mentioned in the table of Cardoso et al., but that were otherwise described in the literature to play a significant role in CRC.

Finally, a systematic search of literature was performed, with Pubmed as main database, using the following search terms: the name of the protein + “immunohistochemistry” + “colorectal cancer”, and the name of the protein + “imaging” + “colorectal cancer”, or variations of these terms, from 1985 until May 2010.

TARGET SELECTION: INTRODUCING TASC

A tumor biomarker can be defined as a distinguishable component present on the tumor cell or secreted by a tumor cell to the surrounding stromal tissue. Such a biomarker is often a target in biological interactions, e.g. the combination of CXCR4 as target of SDF-1. Alternatively, a biomarker can be used as a target for a synthetic substrate, which can be a single molecule, antibody, et cetera. Such a substrate can be conjugated to a diagnostic or imaging agent or a drug for clinical application purposes.

To our best knowledge, a scoring system to identify the most ideal target characteristics has never been explicitly described nor developed or even validated. However, a number of favourable target features can be logically extracted from literature so far. Based on these characteristics, we propose a novel scoring system for target selection in particular for imaging purposes, the Target Selection Criteria (TASC).

The TASC score is based on the seven most favourable target characteristics which are granted points if it applies to the marker. (Table 1) These characteristics are: I) extracellular biomarker localization, either on the cell membrane or in close proximity of the tumor cell; II) expression pattern; III) tumor to healthy tissue ratio (T/N); IV) percentage of

positive tumors; V) reported successful use of the biomarker in *in vivo* imaging studies; VI) enzymatic activity and VII) internalization. (Figure 2)

We will briefly explain these seven individual characteristics:

- I A target must be easily accessible by an agent, administered either systemically or intraperitoneally. For effective targeting, as little possible barriers should be between the agent and its target. As a consequence, most conveniently the marker is present on the cell surface. Alternatively, expression of the target in the extracellular tumor matrix may also be adequate for imaging purposes. In our opinion, the extracellular localization of the target, either membrane-bound or in close proximity to the tumor cell, is one of the most important factors and is therefore weighted substantially in the TASC system. Extra points are given to a cell membrane bound target, since it is expected that membrane bound targets more specifically emit signal from the tumor cell than soluble targets.
- II In the best scenario, the target is expressed by all tumor cells. However, in reality this is very rare, since cancer cells have the reputation of being heterogenic.¹⁷ Also acceptable is a marker that is evenly distributed throughout the tumor tissue. High sensitivity to detect all tumor tissue is essential, therefore this factor also has a significant power in TASC.
- III Expression of the biomarker should be minimal in normal tissue. In modalities like Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT) scanning, a tumor to healthy cell (T/N) ratio of >10 is considered sufficient.¹⁸ In fluorescence imaging a minimal T/N ratio has not yet been described, but is expected to be comparable to the prementioned modalities, based on its detection sensitivity and specificity.
- IV It is highly preferable that the use of a particular biomarker is of value for large patient populations, rather than only small groups of “special” patients. Overexpression of the target in the vast majority of patients increases clinical applicability of a tumor-targeted agent.
- V Previous use of a biomarker in *in vivo* imaging indicates suitability of the marker for imaging purposes in other diseases, in this case CRC.
- VI Although not an absolute condition for a target, (extracellular) enzymatic activity in and around the tumor tissue offers the possibility of applying locally activated imaging agents, so called “smart-probes”, increasing the signal-to-background ratio.¹⁹
- VII It is reported in the literature that internalization of the probe-target complex in the tumor can lead to intracellular accumulation of the imaging agent, which improves the signal and leads to a more optimal T/N ratio.²⁰ For this reason internalization is granted points in the selection criteria.

Selecting a target that meets up to all of these conditions is challenging. In most cases, it is not necessary to meet all criteria.

A total score of 21 or 22 implies that a marker has a high potential for use as a target for imaging tracers *in vivo*. If a marker scores ≥ 18 , it is considered to be a potential target. Markers with a score < 18 seem less suitable for targeted imaging modalities and requires more research to evaluate its potential.

POSSIBLE TARGET CANDIDATES IN COLORECTAL CANCER

It is well known that it can be difficult to distinguish cancer cells from its normal surroundings, because of the many similarities between malignant cells and normal cells. Furthermore, tumors are mutually heterogenic. However, what most cancer cells have in common and what separates them from normal cells is uncontrolled growth, resulting in a high nutritional uptake. An alternative property is the ability to invade normal tissue and metastasize. In this respect it is not surprising that the potential targets presented in this review support these phenotypic characteristics. The biomarkers reported for CRC can roughly be divided into the following groups:

- Proteins necessary for high cancer cell metabolism and proliferation rate: Epidermal Growth Factor Receptor (EGFR), Folate Receptor- α (FR- α), Transforming Growth Factor (TGF), Vascular Endothelial Growth Factor (VEGF).
- Proteins with regulatory functions in the extracellular matrix: carbonic anhydrase (CA) IX, collagen, Matrix MetalloProteinases (MMPs), osteonectin (SPARC).
- Cell adhesion and signaling molecules: Cadherin 3, CarcinoEmbryonic Antigen (CEA), CD44, CXC Chemokine Receptor (CXCR)4, Epithelial Cell Adhesion Molecule (EpCAM), Integrins.
- Cytokines / chemokines and their corresponding receptors, involved in metastasis: CXCR1, CXCR2, CXCR4, CXC chemokine ligands (CXCLs).
- Miscellaneous: Cathepsin, inducible Nitric Oxide Synthase (iNOS), Mucin 1 (MUC1), neutrophil gelatinase-associated lipocalin (NGAL) also called Lipocalin-2 (LCN2), Tumor-Associated Glycoprotein 72 (TAG-72).

These potential targets are summarized in Table 2. As is shown in this table, several potential target candidates can be identified; however currently a limited number of matching clinically approved agents are available for application in humans (Table 3).

Some targets have T/N ratio of < 10 . However, it should be noted that some targets internalize the imaging agent more rapidly in tumor cells compared to normal cells.¹⁸

This leads to an accumulation of the conjugated imaging agent, which may compensate the signal for the lower T/N ratio, as with FDG-PET imaging.^{18, 20}

WHICH BIOMARKERS MEET THE TARGETING CRITERIA?

When applying the proposed TASC score (Table 1) to the biomarkers mentioned in Table 2, not all requirements can be objectified by data from literature. Most often, expression rates and pattern are unknown, therefore it would be interesting to focus future research

on target-finding on these aspects. The following six targets have a score above 17 points and can therefore be considered most promising in CRC: Epithelial Cell Adhesion Molecule (EpCAM) (20 points); Chemokine Receptor 4 (CXCR4) (20 points); Mucin 1 (Muc1) (18 points); Matrix Metalloproteinases (MMPs) (18 points); Epidermal Growth Factor Receptor (EGFR) (20 points); and Carcinoembryonic Antigen (CEA) (19 points). In this section, we discuss these targets in more detail, including the current status of these biomarkers in targeted imaging.

Vascular Endothelial Growth Factor A (VEGF-A) scores 17 points, which implies less potential as a target. However, given the extensive experience in VEGF-A-targeted imaging, this biomarker was nevertheless considered to be promising and is therefore given attention in this section.

EPITHELIAL CELL ADHESION MOLECULE

Epithelial Cell Adhesion Molecule (EpCAM) is a cell surface receptor, which is involved in cell adhesion and is expressed on most epithelial cells. EpCAM is upregulated on several epithelial cancers, including CRC. Expression of EpCAM in CRC is more than 80%.²¹⁻²³ Paradoxically, higher expression of EpCAM on tumor cells is associated with increased tumor cell migration.²³ Eder et al. successfully imaged EpCAM-expressing tumors in mice, using an antibody fragment targeting EpCAM conjugated to radionuclide, for PET-imaging.²⁴

Edrecolomab and Catumaxomab are clinically approved antibodies directed at EpCAM (Table 2), and tested for therapeutic use. However so far no obvious therapeutic advantage has been reported for these agents.²⁵⁻²⁸ To our best knowledge, these antibodies have not yet been used for *in vivo* imaging of EpCAM. When applying TASC to EpCAM; the total score of 20 points comes about as followed: EpCAM is cell membrane bound (5 points), diffusely upregulated (4 points), has a high T/N ratio (3 points), is upregulated in more than 79% of the CRC patients (5), has been previously imaged with success *in vivo* (2) and is able to internalize a compound (1 point).^{21, 22, 29}

CXC CHEMOKINE RECEPTOR 4

CXC Chemokine Receptor 4 (CXCR4) is a cell surface receptor involved in homing of hemopoietic stem cells and lymphocytes to the bone marrow, but it is also associated with metastatic spread in several types of cancer, including CRC. CXCR4 is expressed in approximately 70% of the colorectal tumors.³⁰

Imaging of CXCR4 has recently attracted attention of many different research groups. Nimmagadda et al. reported imaging of CXCR4 in tumor bearing mice using a radionuclide-labeled anti-CXCR4 monoclonal antibody (mAb) using SPECT/CT scanning.³¹ ³² Recently, the same group also succeeded in imaging CXCR4 expressing tumors in mice with the use of AMD3100, a clinically approved molecule that selectively binds to CXCR4 (Table 3), conjugated to a radionuclide.³² AMD3100 is a clinical approved agent which is most promising in harvesting hemopoietic stem cells from the bone marrow.

Alternatively, CXCR4-targeting peptides conjugated to a radionuclide or a fluorophore, have been reported.^{33, 34} Misra et al. labelled stromal derived factor-1alpha (SDF-1alpha), a ligand of CXCR4, to a radionuclide, for myocardial infarction imaging purposes.³⁵ When applying TASC, CXCR4 is granted 20 points based on its expression in CRC.

VASCULAR ENDOTHELIAL GROWTH FACTOR-A

Vascular Endothelial Growth Factor (VEGF) is an epithelial growth factor that is most extensively known for its ability to induce angiogenesis. Angiogenesis in turn is considered one of the primary markers in tumor diagnostics.³⁶ There are 4 VEGF's, namely VEGF-A, B, C and D. VEGF-A is the most important subtype. When tumor cells become hypoxic, VEGF-A expression is upregulated.³⁶ VEGF-A is partly membrane bound, but also diffuses through the interstitial cell space. The latter potentially limits broader use as a target. However, the highest VEGF-A concentrations are observed close to the source of expression, inducing the creation of new blood vessels to the hypoxic tumor areas.³⁷ VEGF-A is upregulated in more than 56-78% of all colorectal tumors.^{38, 39}

Multiple groups have successfully imaged VEGF-A expression in tumors induced in animals using a VEGF-A antibody conjugated to an imaging agent. Imaging has most commonly been performed with bevacizumab (Avastin®, Roche), a clinically approved therapeutic anti-VEGF-A monoclonal antibody which was made suitable for imaging by conjugation to a radionuclide.⁴⁰⁻⁴³

In clinical imaging studies Scheer et al.⁴⁰ did not find a significant correlation between VEGF-A expression and a positive SPECT signal, which may imply that the used tracer was not specific enough. Furthermore, a study in melanoma patients with bevacizumab conjugated to a radionuclide by Nagengast et al. yielded more promising results.⁴⁴

When applying TASC to VEGF-A, a total of 17 points are granted. This low score is mainly caused by the fact that the largest proportion of VEGF-A is not membrane bound; and by the expression in a relatively low percentage of patients with CRC. However, because of the recent results in various imaging modalities, as described above, VEGF-A can be considered a potential target for future imaging purposes and is therefore worth to be included in this overview.

MUCIN I

Mucin 1 (Muc1) is a cell surface receptor which plays a role in protection and lubrication of epithelial surfaces in luminal structures. This receptor is also involved in signal transduction in cell adhesion and anti-adhesion mechanisms. Overexpression of Muc1 is often found on malignant cells. In CRC, Muc1 is expressed on approximately 50% of the tumors.^{45, 46}

Different groups have successfully imaged Muc1 in tumor-bearing mice using muc1-targeted monoclonal antibodies or aptamers conjugated to a radiopharmaceutical.⁴⁷⁻⁵³ The use of monoclonal antibodies directed to Muc1 conjugated to a radionuclide has already been described in patients with bladder cancer and pancreatic cancer.⁵⁴⁻⁵⁶ Medarova et al.

described the use of a dual modality imaging agent, by conjugating a Muc1-targeting peptide to fluorophore Cy5.5 for fluorescence imaging, and to iron oxide nanoparticles for Magnetic Resonance (MR) imaging. This probe was tested in mice bearing human pancreatic cancer with good imaging results for both modalities.⁵⁷ Muguruma et al. proved the ability to endoscopically detect tumors by using a fluorescent antibody-based tracer targeting Muc1, in freshly resected specimens of gastric cancer.⁵⁸ A different approach for tumor imaging is a two-step pre-targeting technique using a bispecific antibody. An antibody directed to both Muc1 and the used radiopharmaceutical is administered upon which the radiopharmaceutical is administered subsequently. The radiopharmaceutical binding site of the circulating antibody can be blocked, thus yielding a higher tumor to background ratio.⁵⁹ Promising results were obtained in breast cancer patients with bispecific antibody-based PET scanning.⁶⁰

The total TASC-score for Muc1 in CRC is 18 points.

CARCINOEMBRYONIC ANTIGEN

CarcinoEmbryonic Antigen (CEA) is a glycoprotein which plays a role in cell adhesion. In healthy adults hardly any CEA is found, however, CEA is strongly expressed in CRC (more than 90%)⁶¹⁻⁶⁴ and is one of its best studied tumor markers. CEA is also measurable in blood, but by far the highest concentration of CEA is found at the tumor site. CEA imaging using a CEA-directed antibody or antibody fragment conjugated to a radionuclide, has extensively been described in animal studies and in patients, without showing disadvantages of having simultaneous high serum and tumor CEA levels.⁶⁵⁻⁷⁰ Yazaki et al. fused CEA-antibody fragments conjugated to a radionuclide to albumin for a more specific tumor uptake.⁷¹ ^{99m}Tc-arcitumomab is a commercially available antibody fragment directed to CEA conjugated to Technetium-99m, which is used in the CEA-scan. However, in comparison to FDG-PET, ^{99m}Tc-arcitumomab offers little convincing advantages in the detection of CRC.^{72, 73} The use of CEA-antibody fragment based radiotracers for guided surgery has also been described.^{68, 70, 74}

As well as in Muc1 targeting, the two-step pre-targeting system using a bi-specific antibody has been described in animal studies and in patients for CEA.^{75, 76}

Few studies are available on fluorescence imaging for targeting of CEA. Fidarova et al. describe the use of an anti-CEA mAb conjugated to a fluorophore for detection of metastatic CRC in mice.⁷⁷ Kaushal et al. showed the use of an anti-CEA mAb conjugated to a fluorophore, in intra-operative detection of colorectal tumor deposits, with good *in vivo* results.⁷⁸ When applying TASC to CEA in CRC, the total score is 19 points.

MATRIX METALLOPROTEINASES

Matrix MetalloProteinases (MMPs) are zinc- and calcium-dependent endoproteases, which are upregulated in the tumor environment and are capable of degrading proteins in the extracellular matrix. MMP's are upregulated in 30-95% of colorectal tumors, depending on the type of MMP (Table 2).^{45, 79-82}

Several groups have targeted MMPs *in vivo* by using fluorescent or radiolabeled specific MMP-inhibitors.⁸³⁻⁸⁶ One study reports using a radiolabeled mAb for *in vivo* targeting of MMP1, an MMP subtype.⁸⁷ Because MMPs have proteolytic activity, this target is ideal for activatable probes. The advantage of activatable probes is that they greatly reduce background signal. Several studies demonstrate the *in vivo* use of proteolytic beacon coupled to a fluorophore, which emits a signal after cleavage by MMP.^{88, 89} MMPsense™ is a commercially available MMP-dependent activatable fluorescent probe, successfully tested in *in vivo* models.⁹⁰ Veisheh et al. describe the *in vivo* use of chlorotoxin, a small peptide derived from snake venom that interacts with MMP2, conjugated to the fluorophore Cy5.5, for potential intra-operative imaging.⁹¹ Lepage et al. synthesized a contrast agent containing gadolinium chelate, which is cleaved by MMP. Upon cleavage, this agent is less soluble in water and remains at the tumor site. Good *in vivo* results have been demonstrated for MR imaging using this protease-modulated contrast agent (PCA).⁹²⁻⁹⁴ Tsien et al. developed activatable cell penetrating peptides (ACPPs) which enter the cell after cleavage by MMP. The ACPPs were labelled with Cy5.5 for fluorescence imaging, or with gadolinium chelate for MR imaging, or both for dual imaging.^{95, 96} These ACPPs were further improved by conjugation to large molecule dendrimers, which improved tumor uptake and thus the emitted signal.^{97, 98}

MMPs are granted an average of 18 points in CRC when applying TASC, depending on the subtype.

EPIDERMAL GROWTH FACTOR RECEPTOR

Epidermal Growth Factor Receptor (EGFR) is a cell surface receptor involved in processes such as cell proliferation, differentiation, adhesion and migration. EGFR is upregulated in different types of cancer, including skin, breast, ovary, bladder, prostate, kidney, head and neck, and non-small-cell lung cancers.^{99, 100} In colorectal cancer EGFR is upregulated in approximately 80% of the tumors.^{101, 102}

EGFR has been extensively imaged by radionuclide or fluorophore conjugated antibodies. Most often, cetuximab, a clinically approved anti-EGFR antibody, is used.¹⁰³⁻¹⁰⁷ In 1994, Dadparvar et al. administered radionuclide labelled anti-EGFR antibodies to patients with intracranial neoplasms, for SPECT scanning.¹⁰⁸ Although promising results were obtained, to our knowledge no sequel was given to this radiopharmaceutical. Also, a few studies describe the use of panitumumab *in vivo*, which is a second clinically approved antibody directed at EGFR.^{109, 110} Variants using antibody fragments or affibodies have been described in animal studies.^{111, 112}

Alternatively, epidermal growth factor (EGF), the natural ligand of EGFR, is also used *in vivo* as imaging agent, conjugated to mainly fluorophores or quantum dots.¹¹³⁻¹¹⁵ Goetz et al. describe a fluorescent anti-EGFR antibody capable of imaging human CRC tissue, which is not only successful *in vivo* imaging results, but also potentially useful in endoscopy.¹¹⁶ Hama et al. describe an alternative two-step pre-targeting model, using nonfluorescent biotinylated cetuximab as first antibody, followed by a neutravidin-BODIPY-FL

fluorescent conjugate. The latter binds to the first antibody by a neutravidin-biotin binding.¹¹⁷ The concept was tested *in vivo* in a PC model. Ten-fold signal amplification was found, leading to high tumor-to-background ratios and good detection of lesions as small as 0.8 mm.

The TASC score of EGFR in CRC adds up to 20 points.

DISCUSSION

TASC needs to be validated in other cancer types and adjusted where necessary.

It should be pointed out that TASC is designed as a directive which can help to gain objectivity and extra insight in target selection. Future validation studies and adjustments will to our opinion improve TASC to make it broadly applicable. Immunohistochemical analysis of collected tumor specimens is a relatively easy way to determine applicability of a target. In the case of a promising target, further validation is needed by testing a target-directed imaging probe *in vitro*, for proof of concept and specific binding, and subsequently in appropriate tumor mouse models.

Expression of a target may depend on tumor stage. For example, CXCR4, EGFR and VEGF are associated with more advanced tumor stages and metastasis in CRC.¹¹⁸⁻¹²⁰ However, MUC1 is also generally expressed in T1 CRC tumors.⁴⁶ Such a target may also be of value in early CRC detection.

CONCLUSION

In peritoneal carcinomatosis (PC) of colorectal origin, tumor-targeted imaging may yield better diagnostic and therapeutic results. A large number of tumor biomarkers are up-regulated in CRC. However, there is no objective system for selecting their clinical applicability in targeted imaging applications. In this review we introduce a novel scoring system for target selection for imaging purposes, the TArget Selection Criteria (TASC). When applying TASC to biomarkers for CRC, we found that the most potent targets for imaging are CXCR4, VEGF-A, Muc1, MMPs, EGFR, EpCAM and CEA based on their scoring. Clearly, the ideal target for imaging purposes does not exist, moreover by using the TASC system we propose a novel guideline in the field of tumor-targeting for selecting appropriate targets for imaging purposes.

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TArget Selection Criteria (TASC) scoring system			
	Characteristics		Score
I	Extracellular protein localization	Bound to cell surface (receptor)	5
		In close proximity of tumor cell	3
II	Diffuse upregulation through tumor tissue		4
III	T/N ratio > 10		3
IV	Percentage upregulation in patients	>90%	6
		70-90%	5
		50-69%	3
		10-49%	0
V	Previously imaged with success in vivo		2
VI	Enzymatic activity		1
VII	Internalization		1
Total: maximum 22			
Potential target ≥ 18			

Table 1. The TArget Selection Criteria (TASC). A biomarker is granted points for seven factors (I-VII). A total score of ≥ 18 indicates that the biomarker is potentially suitable for tumor-targeted imaging purposes.

TASC-item:	I	II	III	IV	V	VI	VII	TASC-score	Ref.
	Extracellular: membrane-bound or secreted	In close proximity of tumor cell	Pattern of upregulation by tumor tissue	T/N ratio	Percentage patients with positive colorectal tumors	Previously imaged	Enzymatic activity	Target-mediated internalization	
CA IX	Membrane-bound	Yes	Focal as well as diffuse	High	~47%	Animal experiment (121, 122)	Yes	Yes (123)	11 124-126
Cadherin 3	Membrane-bound	Yes	Diffuse	High	Probably high; no percentage known	No	Not described	Not described	15 127
Cathepsin B, D	Mostly secreted; partly membrane-bound	Mainly	Diffuse, but most expression at invasion front	High	~60%	Animal experiment (128-130)	Yes	Not described	16 45, 82, 131
CD44	Membrane-bound	Yes	Diffuse	~1.4	~50%	Animal experiment (132)	Not described	Yes (133)	16 134, 135
CEA	Partly membrane-bound; partly secreted	Mainly	Diffuse, not homogenous	>60	>90%	In patients (69, 136, 137)	Not described	Yes (138, 139)	19 61-64
COL11A1	Secreted	Yes	Unknown	Unknown	Unknown	No	Not described	Not described	3 140
CXCL1,5	Secreted	Unknown	Unknown	High	Unknown	No	Not described	Not described	3 30, 141
CXCR1	Membrane-bound	Yes	Diffuse, mainly in primary tumor	High	~55%	No	Not described	Yes (142, 143)	16 30
CXCR2	Membrane-bound	Yes	Diffuse, mainly in primary tumor	High	~60%	No	Not described	Yes (142, 143)	16 30
CXCR4	Membrane-bound	Yes	Diffuse, more expression in	High	~70%	Animal experiment	Not described	Yes (144)	20 30

EGFR	Membrane-bound	Yes	metastases	Diffuse	Unknown, probably high	~80%	(32) Animal experiment (111, 114, 145-147)	Not described	Yes (148)	20	101, 102
EpCAM	Membrane-bound	Yes	Diffuse	Diffuse	High (own data)	>79%	Animal experiment (149)	Not described	Yes (29)	20	21, 22
Folate receptor alpha	Membrane-bound	Yes	Diffuse, little strong expression	Diffuse, little strong expression	High	~40%	In patients (150, 151)	Not described	Yes (152)	15	153
Galectin 3	Partly membrane-bound, partly secreted	Yes	Diffuse, but not homogenous	Diffuse, but not homogenous	High	65-95%	Animal experiment (154, 155)	Not described	Yes (156)	13	21, 157, 158
iNOS	Mainly intracellular	Yes	Diffuse	Diffuse	High	~78%	Animal experiment (159)	Yes	Is already intracellular	16	160, 161
Integrins	Membrane-bound	Yes	Diffuse	Diffuse	Unknown, but imaging T/N ratio >5 (162)	~60%	In patients (163, 164)	Not described	Yes (165, 166)	15	167, 168
MMP1, 2, 3, 7, 9	Mainly secreted	Yes	Diffuse	Diffuse	Moderate to high	30-95%	Animal experiment (88, 89, 91, 169)	Yes	Not described	18	45, 79-82
Muc1	Membrane-bound	Yes	Diffuse, more expression in larger tumors and lymph node metastases	Diffuse, more expression in larger tumors and lymph node metastases	High	~50%	In patients (54-56, 60)	Not described	Yes (170)	18	45, 46
NGAL (LCN2)	Secreted	Mainly	Diffuse	Diffuse	High	~75%	In vitro (171)	Not described	Not described	15	81, 171
Osteonectin (SPARC)	Secreted	Yes	Diffuse	Diffuse	High	High, no percentage known	Animal experiment (172)	Not described	Not described	17	81

TAG-72	Partly membrane-bound; partly secreted	Mainly	Not diffuse	High	46-98%	In patients (173, 174)	Not described	11	175-177
TGFBI	Secreted	Mainly	Unknown	High	Unknown	No	Not described	6	178
VEGF-A	Partly membrane-bound, partly secreted	Mainly	Diffuse, more expression in metastases	High	56-78%	In patients (40, 44)	Not described	17	38, 39

Table 2. Proteins upregulated in colorectal cancer. Under ‘previously imaged’ (item V) only the most advanced research is mentioned. For each biomarker, the final TArget Selection Criteria (TASC) score is given, based on the characteristics as explained in Table 1.

Target	Clinically approved ligand	In clinical trial
EGFR	Cetuximab, Panitumumab, Nimotuzumab	Necitumumab, Zalutumumab
EpCAM	Edrecolomab, Catumaxomab (anti-EpCAM x anti-CD3)	Adecatumumab, Tucotuzumab
CXCR4	AMD3100	BKT-140, AMD11070, MSX-122
VEGF	Bevacizumab, Ranibizumab	
Folate receptor-alpha	Folate	
CEA	Arcitumomab, altumomab	
Muc1	Pemtumomab	90Y-hPAM4
Integrin		MoaB PF-04605412 (mAb against $\alpha 5\beta 1$ integrin), Etaracizumab (mAb against $\alpha_v\beta_3$ integrin)

Table 3. Clinically approved ligands for the biomarkers mentioned in Table 2.

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